Antimicrobial effect of grape seed extract on planktonic cells and biofilms of *Campylobacter* spp.

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Introduction

***Campylobacter* spp.** is a leading cause of bacterial gastrointestinal diseases worldwide, and chicken meat representing the main source of human infections. Its ability to overcome normally lethal environments, despite its fastidious nature and oxygen-sensitivity, is attributed to its **biofilm forming capability**. Utilization of natural plant extracts, such as the polyphenol-rich **Grape Seed Extract (GSE),** has attracted more attention in the recent years due to its health benefits and wide spectrum of antibacterial activity. Major public health concern regarding food preservation, is the occurrence of **sublethally injured cells**, which are susceptible to recovery, developing stress adaptive responses, and responsible for serious limitations in routine microbiological surveillance methods, such as the underestimation of contamination levels and the assumption of false negative results.

Objectives

The objectives of the present study were to assess (i) the occurrence of sublethal injury in *Campylobacter jejuni* cells after exposure to different GSE concentrations, (ii) a potential relationship between membrane damage and loss of cell viability, (iii) the biofilm-forming abilities of different *Campylobacter* strains on different surfaces and (iv) the GSE efficacy against biofilm cells.

Materials & Methods

Sublethal injury & Membrane damage

- ***Campylobacter jejuni* LP1,** a clinical isolate (stationary cells)
- Exposure to **GSE at 500 and 2100 mg GAE/L** (Total phenolic content by Folin-Ciocalteu) in Brucella Broth (BB). Incubation at 42ºC under microaerophilic conditions (85% N_2, 10% CO_2, 5% O_2)
- Recovery media: Müeller Hinton Agar (MHA) and the selective Modified Charcoal Cefoperazone Deoxycholate (MCCD) agar

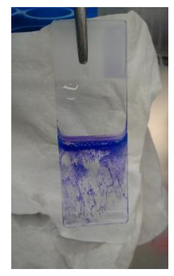
- **Sublethal Injury**

$$\%\ SI = \left(\frac{Counts\ on\ non\ selective\ \ agar\ - Counts\ on\ selective\ \ agar}{Counts\ on\ non\ selective\ agar} \right)$$

- **Membrane damage**: Propidium Iodide staining and TEM visualization

Biofilm-forming ability & GSE inactivation

- ***C. jejuni* NCTC 11168** (commercial strain)
- ***C. jejuni* CC1 and CC2,** isolates from a poultry production plant and a chicken at retail store
- ***Campylobacter coli* LP2**, a clinical isolate (stationary cells)
- Glass, stainless steal and polystyrene surfaces. Incubation at 42ºC under aerobic conditions for 48 h
- **Biofilm formation**: Staining with crystal violet
- **Exposure to GSE at 2100 mg GAE/L**. Detachment of biofilm cells with Trypsin-EDTA and recovery on MHA



Results and Discussion

Sublethal injury in GSE-treated cells

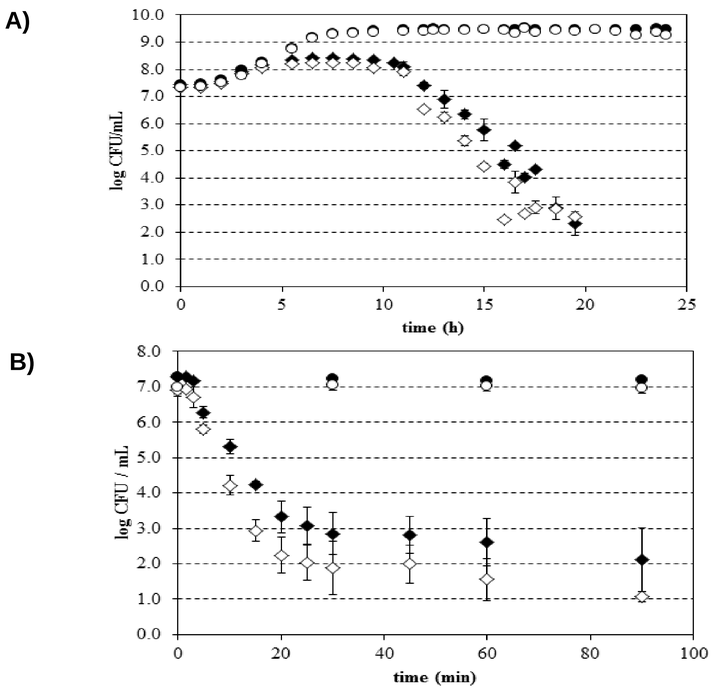


Figure 1, Survival curves of *C. jejuni* in BB supplemented with GSE at (A) 500 and (B) 2100 mg GAE/L (♦,◇) and control in BB (●,○): Viable counts in MHA (solid) and MCCD (hollow).

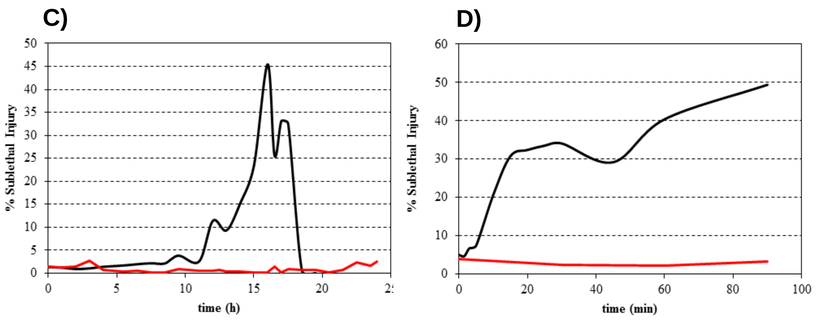


Figure 2. Sublethal injury after exposure to GSE at (C) 500 and (D) 2100 mg GAE/L (black). Control in BB (red).

Membrane damage in GSE-treated cells

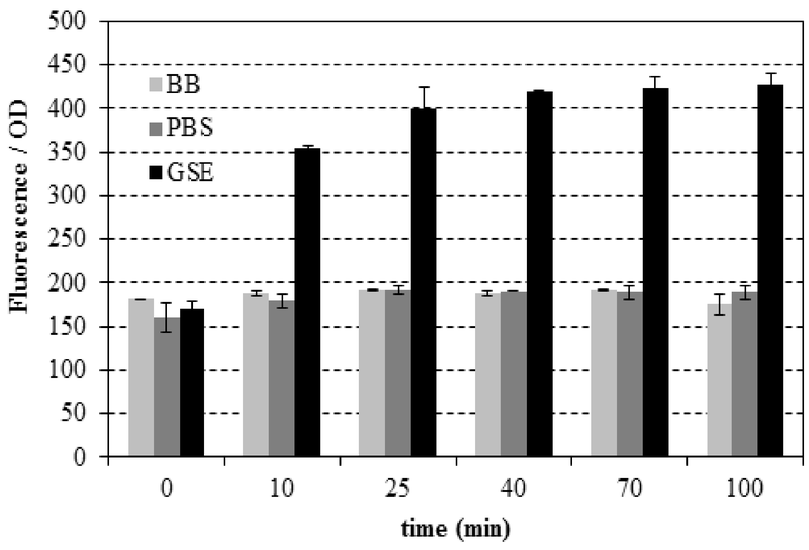


Figure 3. Permeability of *C. jejuni* cells to Propidium Iodide after exposure to GSE. Control in PBS and BB.

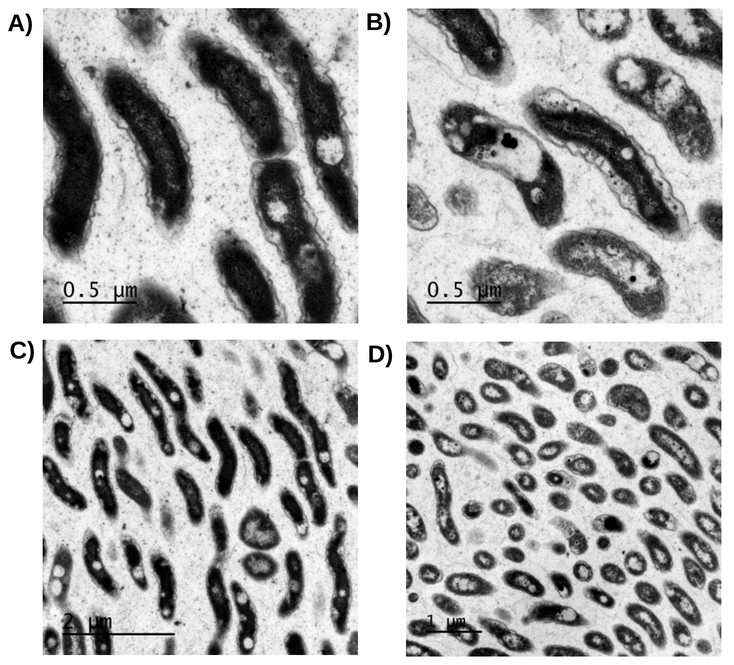


Figure 4. TEM micrographs of GSE-treated cells (B and D) and control (A and B).

Biofilm forming-ability

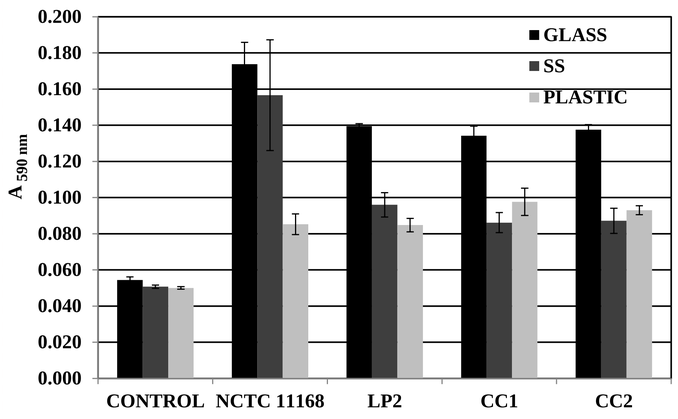


Figure 5. Biofilm-forming ability of *Campylobacter* strains

GSE efficacy against biofilm cells

Table 1. Concentration of biofilm cells (NCTC 11168) on glass surface after exposure to GSE

GSE exposure time (h)	Concentration of biofilm cells (CFU / glass coupon)	
	Treated cells	Control
0	$1.1 \times 10^6 \pm 3.8 \times 10^5$	$1.1 \times 10^6 \pm 3.8 \times 10^5$
0.5	$6.3 \times 10^5 \pm 2.4 \times 10^5$	$1.1 \times 10^6 \pm 1.2 \times 10^5$
1	$3.1 \times 10^4 \pm 1.1 \times 10^4$	$1.0 \times 10^6 \pm 1.1 \times 10^5$
3	ND	$8.6 \times 10^5 \pm 1.7 \times 10^4$

Conclusions

GSE inhibited planktonic growth in a dose dependent-manner, exposure times being critically reduced with increasing GSE concentrations. A mechanism of cumulative damage, triggering lethal instead of SI, is suggested from SI distribution profiles and attributed to a loss of membrane integrity. All strains formed biofilms on the multiple surfaces, especially on hydrophilic glass, and sessile cells exhibited greater GSE resistance than planktonic cells. This information is essential for the optimal design of combined hurdle technologies that efficiently combat *Campylobacter* colonization of foods.

References

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Acknowledgements

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